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Original Article

Partial substitution of sucrose by non-nutritive sweeteners in sour orange marmalades: effects on quality characteristics and acute postprandial glycemic response in healthy volunteers

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Abstract

Background: Overconsumption of added sugars, particularly refined sugars, has been shown to be associated with adverse health concerns. **Aim:** The present study aimed to elaborate calorie-reduced marmalades with nutritional benefits as well as satisfactory sensory properties, in order to reduce sugar intake without compromising consumers' acceptance. **Materials and methods :** Two formulas of sugar-reduced marmalades were elaborated by substituting 30% of sucrose with different commercial non-nutritive sweeteners: a blend of aspartame-acesulfame-K and sucralose. Physico-chemical, sensory, and microbiological analyses were carried out, in comparison with control sample marmalade. Blood glucose concentrations were determined in 12 healthy volunteers, at 30-min intervals until 120 min after consumption of marmalades. **Results:** Marmalade quality characterization revealed a significant effect of sucrose substitution on dry extract, Brix, reducing sugars, aw, and CIE Lab color parameters, but not on pH and acidity. The microbiological analysis highlighted that marmalades' sanitary quality was in accordance with safety standards. Interestingly, sensory analysis by trained panelists showed that the substitution of sucrose by an intense sweetening substance did not impair the sensory properties. Our data also indicate that consumption of calorie-reduced marmalades significantly reduced acute postprandial glycemic responses in healthy volunteers; this effect was more pronounced with sucralose. **Conclusions**: Taken together, our results showed that the use of sucralose can constitute a relatively healthy choice for food basket of families, in particular for those with high risk of lifestyle-related diseases.

Keywords: Citrus marmalade, Aspartame-acesulfame-K, Sucralose, Calories reduction, Quality, Glycemic response.

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1 Introduction

Sugars are a ubiquitous constituent of food ration and are consumed as a naturally occurring component of several foods and as additions during processing, preparation, or as sweeteners. According to the Organization for Economic Co-operation Development (OECD) and the Food and Agriculture Organization (FAO) statistics [1,2], their consumption has augmented steadily during the last two decades. However, overconsumption of added sugars, especially refined sugars, has long been thought to be associated with adverse health issues, including dental caries, overweight, diabetes, and heart disease[3-6]. The Joint committee of the Food and Agriculture Organization/World Health Organization (FAO/WHO) [7] has recommended that populations should reduce their daily refined sucrose intake to less than 10% of their total energy intake, and ideally less than 5% of their total energy intake. These recommendations have raised the consumers' awareness about the amounts of daily refined sugar intake. Therefore, consumers' interest in low-calorie dieting has waned, and they have returned to watching their calorie intakes [8-9]. Calorie reduced products are industrial foodstuffs with particular beneficial nutritional properties due to a reduction of energy by at least 30%, in comparison with the original food or a similar food [10]. Actually, one of the most successful innovations is replacing sugar or partially replacing sugar with non-nutritive sweeteners into food products. This strategy addresses the consumer desire for no- or low-calorie sweetness. Bulk and intense sweeteners are food additives able to mimic sugar taste and can help to control body weight and glycemia [4]. In addition, they may contribute to reducing the risk of cariogenicity [11]. However, calorie-reduced food products are regarded as products of less organoleptic quality [8]. Consumers have no willingness to sacrifice taste quality for other advantages such as calorie-reduced contents [12]. Determining the best sweetener for a product is then a critical step because a sweetener, beside being safe and compatible with the product, should keep the specific flavor of a sucrose manufactured product [13]. Non-nutritive sweeteners (NNS), or artificial sweeteners, are sweet-tasting, allowing reductions in energy intake without reducing food and drink palatability [14]. They are not involved in the glycemic response because they are not of carbohydrate nature or not absorbed in the small intestine[15]. The non-nutritive artificial sweetener, aspartame (E951) or N-(L-α-Aspartyl)-L-phenylalanine, 1-methyl ester, has

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a clean sugar-like taste, with sweetness 200 to 300 times greater than that of sucrose, and without undesirable bitter or metallic taste [16]. Because of its very low price, this sweetener provides an attractive option for manufacturers. Acesulfame potassium or acesulfame K (E 950) is about 200 times the sweetness of sucrose, and heat stable, so can be utilized in cooking and baking[11]. However, at higher concentration, it has a low bitter after-taste; this is why it is frequently combined with other artificial sweeteners, such as aspartame [16]. The aspartame and acesulfame K salt (E962) both do not only provide the same sweetening capacity as aspartame, but it also improves the stability to heat and minimizes undesirable reactions of aspartame with other food ingredients [11]. However, to this day, the status of aspartame remains controversial, because of the discrepancy of scientific knowledge about the safety of aspartame, in support and against its use [17,18]. In 2013, the European Food Safety Authority (EFSA) [19] has re-evaluated aspartame as a food additive, and has considered that there were no safety concerns at the Acceptable Daily Intake (ADI) of 40 mg/kg body weight/day for the general population. Acesulfame K has been widely approved as a food additive by the European Commission [10] and it is considered as Generally Recognized As Safe (GRAS) by the US FDA (ADI 9 mg/kg body weight/day) [14,16]. Sucralose (E955) is characterized by a gustatory profile close to sucrose and great stability over a wide range of pH and temperature, thus suited for cooking, and mixing with acidic foods [11,16]. In addition, Gwak *et al.* [20] established that both of these intense sweeteners exhibited relatively low bitterness and less persistent flavor duration in mouth which are undesirable sensory characteristics for sweeteners, when compared to Stevia. Interestingly consumption of NNS such as aspartame, acesulfame K, and sucralose has been shown to not increase blood glucose level in healthy adults[15,21-23]. According to the European Food Safety Agency (EFSA) [24], there was a cause-and-effect relationship between the consumption of foods/drinks containing NNS as sucralose instead of sugar, and reduction in postprandial blood glucose responses. Moreover, aspartame, acesulfame K, and sucralose may be relatively inert regarding to perceptions of hunger or fullness [25-26].

Sour orange *Citrus aurantium* L. belongs to the *Rutaceae* family. This tree is now growing throughout the Mediterranean region, including Tunisia; mostly in the Cap Bon region (125.000 trees; 450 hectares) [27]. It is used mainly as a rootstock and also for its flowers. Neroli essential oil is produced from sour orange flowers using hydro-distillation [27]. Uncollected flowers are fertilized and, after maturation, they form fruits called bitter or sour oranges. Fruits have numerous beneficial properties due to their high content of fibers, vitamins, minerals, and natural antioxidants such as limonoids, flavonoids, and coumarins [28-29]. However, because of their intense sourness and bitterness, they are rarely consumed raw. They are transformed into marmalades and orange-flavored liqueurs [30]. Marmalades are produced primarily from clear citrus juices and have fine shreds of peel suspended in the gel [31]. Marmalades are made of a minimum of 200 g of citrus fruits per 1000 g of the finished product [31]. Several studies have developed calorie-reduced citrus jams, but sucrose replacement by sweeteners affected both sensorial and rheological parameters of jams [32-35]. From a technological point of view it is challenging to manufacture marmalades without sugar incorporation because sugars contribute not only to the marmalade sweet taste and sensory characteristics (aroma, texture, and appearance), but also to the microbial (preservation and fermentation), chemical (inversion and caramelization), and physical (crystallization, viscosity, osmotic pressure, hygroscopicity, consistency/bulk, grain size, and distribution) properties [36]. The originality of this work consists in the valorization of *Citrus aurantium* L. (sour orange) in order to formulate low calorie sour orange marmalades using non-nutritive sweeteners. Sour orange peels are used as a source of pectin in marmalade confection [30]. Interestingly, Abid *et al.* [37] and Hosseini *et al.* [38] have shown that this pectin was low in methoxyl, suitable for sugar-reduced marmalades. In fact, jams produced with low methoxy pectin can turn into gel at concentrations of soluble solids ranging from 10-70%, and pH from 2.00 to 6.00 [38].

This work was carried out to formulate low calorie sour orange marmalades using NNS, sucralose, and a commercial blend of aspartame and acesulfame K, and to evaluate the effect of partial substitution of sucrose by these sweeteners on physico-chemical and sensory properties of sour orange marmalades, as well as on acute postprandial glycemic responses in healthy volunteers.

2 Materials and Methods

2.1 Raw material

Fruits of *Citrus aurantium* L var amara were harvested from an orchard located in the Tunisian Cap Bon during January 2017 and 2018. The harvested sour oranges were sorted and resulted in 100 kg of healthy, ripe, medium size, and presenting no lesions fruits that were transported to the laboratory in Tunis. The fruits were stored at a cool and at shady place prior to manufacturing. Aspartame and acesulfame K salt (PubChem CID: 25130065; commercial grade Marque n°1®), and sucralose (PubChem CID: 71485; commercial grade Canderel®) were purchased from a local supermarket (Carrefour® Tunis, Tunisia).

2.2 Manufacture of marmalades

Marmalades formulations were prepared with sugar (control), with substituting part of sugar for either aspartame-acesulfame-K (ASP-ACEK) or sucralose (SUC). Marmalades were produced from a mixture of sour orange juice, pulp and peel, as described by Ellouze *et al.* [30]. In order to prepare the marmalades, the fruits underwent washing, cleaning, and cutting into thin slices. Then, the proper amount of sugar was added and the different components namely juice, peel, and sugar were let to soak. At the end of soaking proper time the NNS substances SUC or ASP-ACEK were added to their respective batches and cooked. All prepared marmalades were packaged in glass jars and sterilized. Three batches were prepared for each formula. Preliminary jam manufacturing assays have shown that desired jam-like soft solid characteristics of marmalades were met by substituting up to 30% of the added sucrose by NNS substances SUC or ASP-ACEK, and by adjusting peel soaking conditions. Three formulas of sour orange marmalades were preserved at room temperature until analyses.

2.3 Physico-chemical analysis

The total acidity of marmalade was determined by the volumetric method [39]. Volatile acidity was done by a preliminary hydro distillation followed by a titration with NaOH in the presence of Phenolphtaleine [40]. Marmalade pH was measured at 20 ± 2 °C [41] using a pH-meter type 3310 JENWAY. The total dry residue was determined by desiccation of the sample at the temperature of 105 ± 2°C until a constant mass, expressed in g/100g [42]. The determination of the insoluble dry residue [43] was carried out by filtration followed by desiccation of the residue on the filter, at 103 ± 2°C during 3 hours. The soluble dry residue or °Brix was determined using a refractometer Abbe Atago 89553 [42]. Dosage of reducing sugars in marmalades formulas was carried out by the method with Dinitro salicylic acid (DNS). Results are expressed using a standard curve prepared previously with solutions of increasing concentrations of glucose and fructose [44]. The total calorie value was calculated by adding up the calories provided by the energy-containing nutrients: protein P, carbohydrates Cb, and fat F, using the following calorie conversion factors for products of vegetable origin: 4.05P+ 4.03Cb + 8.37F [45].

The measurement of water activity was carried out at 25°C by a hygrometer (aw SPRINT TH-500 NOVASINA).

2.4 Microbiological analysis

The sanitary quality of sour orange marmalades was assessed by the enumeration of yeasts and molds in samples according to NF V 08-059 on Sabouraud medium [46].

2.5 Determination of CIE Lab color parameters

Color coordinates CIE L*, a*, and b* were determined by a colorimeter MINPLOTA CR6300. In this coordinate system, the L* value measures lightness, ranging from 0 (black) to +100 (white). The a* value ranges from –100 (green) to +100 (red), and the b* value ranges from –100 (blue) to +100 (yellow). The total color differences (ΔE*) between the control and sugar-reduced samples was calculated from obtained color coordinates by applying:

$$\Delta E^* = [(Lc-Ls)^2+(ac-as)^2+(bc-bs)2]^{1/2}$$

Where Lc, ac, bc are the coordinates of the control sample, and Ls, as, bs, the coordinates of the sugar reduced marmalades [47].

2.6 Sensory analysis

A discriminative triangular test was carried out using a panel of 10 trained panelists [47]. Three coded samples were presented: two samples were identical and one was different, and panelists were asked to identify the one that was different. The triangular tests were carried out with combinations of samples: (C/ASP-ACEK), (C/SUC) and (ASP-ACEK/SUC) [49].

2.7 Measurement of acute postprandrial glycemic responses

Seven men and five women aged 23-29 years, a body mass index of 18.4-24.8 kg/m2, were recruited from the National Institute of Agronomy of Tunisia (INAT, University of Carthage; Table 1). All study participants were healthy and provided written informed consent for this trial, approved by the Department of Agri-food industries board (INAT). All participants were randomly assigned to the marmalade's samples, with the order balanced and were blinded to the sample allocation. After a 10-h overnight fasting, subjects arrived at the Institute, where they completed health questionnaires. Twenty grams of control or sugar-reduced marmalades were served and subjects were given 5 minutes to finish the marmalades. Twenty grams of marmalades were chosen as this is the amount commonly added to bread [48]. The blood glucose levels were assessed before and at 30, 60, 90, and 120 min after the consumption of the test sample, and were determined by a capillary blood glucose analyzer, calibrated before its use (Accu-Chek Active system, Roche). Results were expressed as change from baseline (t=0) of blood glucose levels at 30, 60, 90, and 120 min after jam consumption.

2.8 Statistical analysis

Table 1: Clinical data and experimental fasting glucose levels

Parameters	Value
Number of subjects	12
Age (years old)	26 ± 3
BMI (kg/m^2)	22.8 ± 2.2
Fasting blood glucose (mg/dL)	93 ± 6

Experiments were conducted twice. All measurements were done in triplicate. The statistical analyses consisted of one way or two ways ANOVA tests and were carried out using GraphPad PRISM software (2018). Values of the incremental area under the curve (iAUC) for blood concentration were calculated using GraphPad PRISM software (2018).

3 Results

Table 2 showed the effect of substituting 30% of sucrose by the intense sweeteners on physico-chemical, microbial, and sensory characteristics of marmalades. All samples of marmalades were characterized by a low pH (pH = 2.8) and no significant differences ($p>0.05$) were found in pH, total, and volatile acidity. The contents of dry matter were significantly lower in ASP-ACEK and SUC marmalades when compared to control ($p<0.05$). This variation was accompanied by a decrease in soluble dry matter (°Brix) and reduced sugars in ASP-ACEK and SUC samples, in comparison with the control ($p<0.01$). Therefore, and based on the equation proposed by Southgate and Durnin [45], substituting 30% of sucrose for two intense sweeteners ASP-ACEK and SUC led to a calorie reduction of respectively 33% and 39%. According to European Regulation No 1924 [10], an energy-reduced product must have an energy value reduced by at least 30% in comparison with the original food or a similar food.

As shown in Table 2, marmalade aw significantly increased in low calorie marmalades, differently according to the nature of NNS incorporated into the formula, without affecting fungal loads (<10 UFC).

The substitution of a part of sugar by intense sweeteners influenced the color of marmalades as shown in Table 3. The

control marmalade was characterized by the highest lightness (L*) and yellow index (b*) values, indicating a lighter yellow color, when compared to those of aspartame-acesulfame K and sucralose marmalades. On the other hand, there was a decrease in the red index (a*) in the control marmalade, when compared to ASP-ACEK and SUC ones. The values of total color difference (ΔE*) ranged from 24 to 63.

Triangle tests were performed to determine whether marmalades containing intense sweeteners SUC or ASP-ACEK were perceptually different from the control original marmalade, and whether SUC marmalade was perceptually different from ASP-ACEK marmalade [49]. As indicated in Table 4, no significant difference was found between the marmalades (C/ASP-ACEK), (C/SUC) and (ASP-ACEK/SUC).

by NNS on quality parameters of sour orange marmalades. In fact, partial substitution (30%) of sucrose by NNS induced significant decreases in total dry matter, soluble dry matter (°Brix) and reduced sugars contents, when compared with the control ($p<0.01$). Whereas the parameters: pH, total acidity, volatile acidity, and insoluble dry residue were not affected by the partial sucrose substitution ($p>0.05$).

Marmalades have shown low pH, since they were elaborated from fruits characterized by a naturally high acidity [50]. This pH below 3.8 can help to ensure proper microbiological stability as low pH restrains most microbial strains growth [34]. The lack of differences in pH, total, and volatile acidity of the control and calorie-reduced marmalades, was in agreement with results of El Zalaki & Luh [51] and Ragab [52] showing no difference in acidity between calorie

Table 2: Physicochemical composition, calorie value and microbial quality of marmalades (n=3)

Parameters*	Marmalade formula		
	C	**ASP-ACEK**	**SUC**
pH	2.8±0.0[a]	2.8±0.0[a]	2.8±0.0[a]
Total acidity (g citric acid.100 g^{-1})	0.13±0.01[a]	0.12±0.02[a]	0.13±0.01[a]
Volatile acidity (g citric acid.100 g^{-1})	0.014±0.001[a]	0.014±0.001[a]	0.021±0.009[a]
Total dry matter (g.100 g^{-1})	67.6 ±0.1[a]	58.0±0.7[b]	62.8±0.1[c]
Insoluble dry matter (g.100 g^{-1})	4.6±1.6[a]	5.3±2.1[a]	5.9±0.4[a]
Soluble dry matter (°Brix)	65.0±0.0[a]	59.0±0.0[b]	60.5±0.5[c]
Reducing sugars (g.100 g^{-1})	65.0 ±1.2[a]	43.0 ±1.3[b]	39.4±1.3[c]
Water activity (aw)	0.655±0.001[a]	0.686±0.001[b]	0.660±0.001[c]
Yeasts and moulds (UFC/g)	<10	<10	<10
Calorie value (Kcal/g)	266[a]	176[b]	161[c]

C: control-marmalade; ASP-ACEK: aspartame-acesulfame K marmalade; SUC: sucralose-marmalade
*Results were expressed as mean ± SD of 3 independent determinations
[a, b, c] Different letters in a raw indicate a significant difference between samples ($p<0.05$).

Postprandial glycemic responses were evaluated upon acute consumption of control and calorie-reduced marmalades in healthy volunteers. As shown in Figure 1A, the partial substitution of sucrose by NNS has significantly impacted acute postprandial glycemic response in healthy volunteers. Changes from baseline in blood glucose concentrations were peaked at 30 minutes, and then declined, before returning to baseline at min 60 for calorie reduced marmalades and after 120 min with controls. Two-ways ANOVA analysis revealed a significant difference in glycemic response by treatment (sweetener agent) ($P<0.0001$), and by time ($P < 0.0001$) as well as by interaction between treatment (sweetener agent) and time ($P < 0.0001$). As indicated on Figure 1B, iAUC for blood glucose was statistically different between the control and NNS marmalades ($p<0.0001$). Moreover, the iAUC was lower after consumption of sucralose marmalade, when compared to ASP-ACEK samples ($p= 0.0060$).

4 Discussion

Quality characterization of control and sugar-reduced marmalades has clearly pointed out an impact of partial substitution of sucrose

reduced jams manufactured with sucrose or intense sweeteners.

The dry matter content is crucial in jam quality assessment, since it correlates with the content of nutrients [53]. In fact, sugar increased the content of dry matter [53]. Regarding to soluble dry matter (°Brix) in reduced sugar samples, only the control sample was complying with Codex standards [31] fixing the °Brix of marmalades to 65%. According to CODEX STAN 296 [31], this Standard does not apply to products in respect of which sugars have been wholly or partially replaced by permitted sweeteners. Similar results were found in other studies [34-35, 54-58]. Interestingly, variations in dry and soluble dry matter contents were not accompanied by a variation on the insoluble dry residue. The insoluble dry residue was a component related to the nature of raw material, and not to the type of sweetening substance incorporated, the raw material (juice and peel) of *Citrus aurantium* did not change from one formula to another. The mean values for reducing sugar contents varied in the ranges 39-65 g per 100 g of marmalades. These values are typical for low-sweetened jams [52,58-59]. The control marmalade contained higher amounts of sugars compared to the other jams. The differences in sugar contents between marmalades formulas can mainly result

from different amounts of sucrose used to production of jams, as well as from possible differences in the sugar content of the fruit. Since the calorie reduction was above 30% in accordance with European Regulation No 1924 [10], the marmalades could be classified as low- calorie products.

Marmalades water activity was significantly affected by the nature of the sweetening agent used, in accordance with data from Rubio-Arraez *et al.* [34]. Marmalades aw values were inversely proportional to dry matter contents. As mentioned by Raoult [53], sugars as well as heat treatment could allow the reduction of aw and consequently the risks of contaminations. The high level of moisture supposed a shorter storage time. In order to confirm this hypothesis a storage study of the prepared NNS prepared marmalades is required. Because of low values of Brix (<60 °Brix), calorie reduced marmalades should be stored below 5°C after opening [60]. In fact, the ability of sugar and pectin to bind water can make it unavailable for microbial growth. In general, the minimum aw for most molds is 0.8 [61]. The number of yeasts and molds in marmalades formulas was in compliance with the safety standard [46], indicating the efficacy of marmalades cooking, and sterilization operations [60].

Table 3: Variation of CIE Lab color parameters of control and reduced calorie sour orange marmalades (n=3)

CIE Lab color parameters	Marmalade formula		
	C	ASP-ACEK	SUC
L*	55.37±1.34[a]	15.64±0.88[b]	4.10±0.71[c]
a*	2.63±1.02[a]	4.81±0.47[b]	5.95±0.63[c]
b*	46.78±0.70[a]	22.80±0.20[b]	8.69±0.56[c]
ΔE*	-	24	63

C: control-marmalade; ASP-ACEK: aspartame-acesulfame K marmalade; SUC: sucralose-marmalade

[a, b, c] Different letters in a row indicate significant differences between samples ($p<0.05$)

The color is a very significant sensory parameter that largely influences preference and acceptance of foodstuffs. The substitution of a part of sugar by intense sweeteners influenced the lightness (L*), the red (a*), and yellow (b*) index values. This led to an increase in value of the total color difference (ΔE*), indicating a highly perceptible difference between the samples, by consumers. Similar results were observed by Ragab [52], Rubio-Arraez *et al.* [34] , and Vilela *et al.* [62]. Basu *et al.* [35] have reported a close and direct relationship between color and soluble solids content. This difference was probably due to the effect of heat on sweeteners used during cooking. The color is formed as a consequence of sugars caramelization and Maillard reaction [53]. Indeed, sugars intervene in food reactions especially the formation of colored compounds. In opposition, because of their thermal stability, both acesulfame K and sucralose do not degrade and react with amino acids by Maillard reaction [63]. However, heat supports their hydrolysis [64]. Our results have also highlighted that the nature of sweeteners influenced the color of reduced calorie marmalades. Significant differences in CIE Lab parameters were detected between ASP-ACEK and SUC marmalades. Aspartame has been shown to be able to interact with food components in particular with reducing sugars (Maillard reaction). This interaction could generate color changes on final products [65]. Because of sucralose neutral effect [33], L* and b* color indexes of SUC marmalade were the lowest, compared to sucrose and ASP-ACEK samples. Similarly, Rubio-Arraez *et al.* [34] have reported the impact of the different ingredients on the food system by their concentration or distribution within the different system phases as well as by different component interactions.

The sensory properties of foodstuffs are extremely significant because they determine the product acceptance by consumers. In fact, keeping the sensory quality characteristics in calorie reduced products compared to reference items plays a crucial role in consumers' acceptability [14]. Triangle tests have indicated no significant differences between the marmalades (C/ASP-ACEK), and (C/SUC) as well as between ASP-ACEK/SUC. Consequently, the substituting 30% of the added sugar by two intense sweetening substances SUC and ASP-ACEK did not significantly affect the sensory quality of marmalades. The chemical and color differences between these formulas were not perceptible by the sensory system of trained tasters. Preliminary formulation assays which consisted of varying the amounts of NNS and studying their impact on the marmalades sensorial characteristics specifically have led to establishing the right dose of sweeteners. These data are in agreement with those of Basu *et al.* [35], De Souza *et al.* [13], Markey *et al.* [59], and Stamatovska *et al.* [66] who reported no significant difference in sensory quality between traditional and low-sugar jams.

Our findings of decreased blood glucose concentration and iAUC have clearly shown a significant effect of the partial substation of sucrose by NNS in marmalades on acute postprandial glycemic response in healthy volunteers. Consumption of a jam provokes glucose entrance into blood circulation, triggering the secretion of insulin by pancreatic β-cells [48]. The intake of control product produced a greater and significant increase in incremental blood glucose than calorie reduced marmalade intake, creating lower glycemic responses. The glycemic index of pure sucrose is 87, whereas pure aspartame, acesulfame K, and sucralose have zero glycemic indexes. This was related to the fact that non- nutritive sweeteners are not metabolized in the human body. Steinert *et al.* [67] observed that intra-gastric infusion of sucralose did not influence plasma glucose concentrations. Glucose and fructose are energy metabolism sugars but sweeteners are not. Post hoc analysis revealed significant difference only at t=30 min after consumption of ASP-ACEK and SUC marmalades ($p=0.03$). This was also observed by Bryant *et al.* [26], requiring further exploration. Broadly speaking, intake of food products containing non-nutritive sweeteners produced lower incremental glycemic responses in healthy volunteers, when compared to sucrose and glucose intake [15,21,23].

Accumulative data elucidated a positive correlation between increased postprandial glycemic responses, and the development of chronic metabolic diseases such as obesity, type 2 diabetes, and cardiovascular disease [4,68]. EFSA [24] has concluded that a reduction

of postprandial glycemic responses (as long as postprandial insulinemic responses are not disproportionally increased) may have a beneficial physiological effect. The current study did not show any effect of non -nutritive sweeteners on type 2 diabetes and obesity.

Our study has several limitations. These limitations are related to the assessment of the acute effects of partial substitution of sucrose with non-nutritive sweeteners in marmalades but the long-term effect of the partial replacement should be elucidated. Furthermore, this study was conducted on healthy volunteers, and should be taken cautiously for people with a high risk of lifestyle-related diseases or compromised glucose tolerance [4-5].

5 Conclusions

Sour oranges are a good source of antioxidants but cannot be eaten raw because of their intense sourness. In order to valorize these fruits and to help consumers to reduce their sugar intake, calorie reduced marmalades were developed by substituting a part of sucrose with non-nutritive sweeteners preserving the quality of the product. The physicochemical characterization of marmalades prepared with a sweetening substance revealed a significant impact on quality parameters such as dry matter and Brix, and instrumental color. However, according to the sensory discriminative analysis, the partial substitution of sucrose by a sweetening substance has not impaired the sensory properties of the product. Interestingly, consumption of marmalades produced with aspartame-acesulfame K or sucralose sweeteners reduced acute postprandial blood glucose concentrations in healthy volunteers, compared to sucrose marmalades. Therefore, using of non-nutritive sweeteners in place of sucrose at recommended doses in sour orange marmalades can constitute one of the most effective strategies to decrease sugar intake without compromising sensory quality.

Author contribution: A.B. and H.D. conceived and designed the study, and undertook the literature research. A.B., and I.E. participated in the experiment and data acquisition. A.B. and H.D. performed the data analysis, and carried out the statistical analysis. A.B. and H.D. prepared, reviewed and drafted the manuscript. All authors approved the final version before submission. All authors have read and agreed to the published version of the manuscript.

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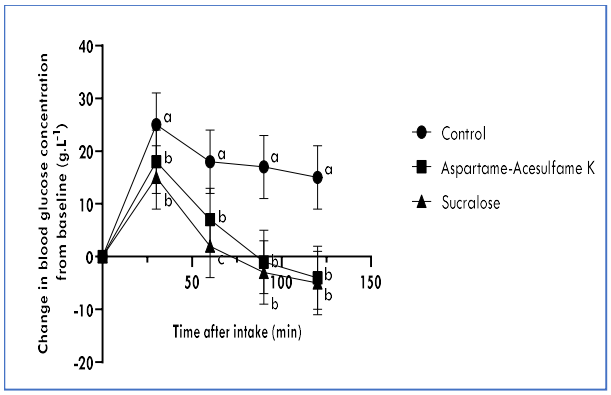
Table 4: Sensory scores of sensory triangle tests for marmalades (n=10)

	Answers to the test		
Panellist number	**C versus ASP-ACEK**	**C versus SUC**	**ASP-ACEK versus SUC**
1	(+)	(-)	(-)
2	(-)	(-)	(-)
3	(-)	(-)	(-)
4	(-)	(+)	(+)
5	(-)	(-)	(-)
6	(-)	(-)	(+)
7	(-)	(+)	(-)
8	(-)	(+)	(-)
9	(+)	(-)	(+)
10	(-)	(-)	(-)
T.C.A	2	3	3
P value	0.896	0.701	0.701

C: control-marmalade; ASP-ACEK: aspartame-acesulfame K marmalade; SUC: sucralose-marmalade. TCA : total comment answer
The sign (+) corresponds to a correct answer (the panelist recognized the different sample out of three)
The sign (-) corresponds to a false answer (the panelist did not distinguish which sample was different).



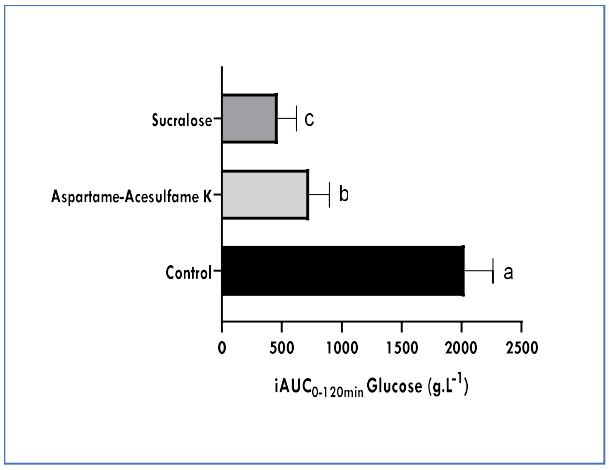


Figure 1: Blood glucose after consumption of the control, ASP-ACEK and SUC marmalades (n = 12) (A) adjusted glucose course, and (B) incremental area under curve (iAUC) for blood glucose concentration. a,b,c Different letters for each time point indicate significant differences between samples ($p<0.05$)

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